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Dinosaurian growth patterns and rapid avian growth rates

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Did dinosaurs grow in a manner similar to extant reptiles, mammals or birds, or were they unique¹? Are rapid avian growth rates an innovation unique to birds, or were they inherited from dinosaurian precursors²? We quantified growth rates for a group of dinosaurs spanning the phylogenetic and size diversity for the clade and used regression analysis to characterize the results. Here we show that dinosaurs exhibited sigmoidal growth curves similar to those of other vertebrates, but had unique growth rates with respect to body mass. All dinosaurs grew at accelerated rates relative to the primitive condition seen in extant reptiles. Small dinosaurs grew at moderately rapid rates, similar to those of marsupials, but large species attained rates comparable to those of eutherian mammals and precocial birds. Growth in giant sauropods was similar to that of whales of comparable size. Non-avian dinosaurs did not attain rates like those of altricial birds. Avian growth rates were attained in a stepwise fashion after birds diverged from theropod ancestors in the Jurassic period.

Attempts to quantify dinosaurian growth rates have focused on the analysis of bone microstructure, but because dinosaur bones are uniquely composed of tissues with both slow-growing reptilian and rapid-growing avian/mammalian attributes, the results have been inconclusive^{3–11}. In addition, the same bone tissue can form at

substantially different rates^{12,13} and the relationship between localized measures of tissue formation rates and overall body growth are untested for nearly all skeletal elements¹⁴. Broader studies have combined histological age assessments with size indices, but lacked reliable means to evaluate developmental body mass¹⁵.

In most extant animals, mass changes with respect to age show sigmoidal patterns¹⁶. Standardized comparisons of maximum growth rates among phylogenetically and morphologically divergent taxa can be made using values from the exponential stage of development^{17,18}. Analyses of exponential growth among the major groups of extant vertebrates indicate that rates absolutely increase with respect to body mass and that each clade has characteristic rates¹⁷.

To compare whole-body growth rates between the Dinosauria and extant vertebrates, similar quantified data are needed. This requires growth series representing the full range of dinosaur size, shape and phylogeny, and accurate age and mass assessments at all ontogenetic stages. To our knowledge, no study of growth rates has fully met these requirements. However, recent merging of bone histology and scaling principles has provided the requisite tools and data for a single dinosaur (*Psittacosaurus mongoliensis*)¹⁴. At last, the pieces are in place to assess how dinosaurs really grew.

We studied genera of non-avian dinosaurs that span a diversity of phylogeny, size and shape (Fig. 1). We included representatives from most major dinosaurian clades, as well as taxa ranging in size from tiny bipedal theropods to enormous quadrupedal sauropods. Notably, our sample also spans nearly the entire temporal range for non-avian dinosaurs, with the inclusion of taxa from the Early Jurassic to Late Cretaceous periods. We previously generated histological samples and longevity data for two dinosaurs (*Apatosaurus excelsus*⁶ and *Psittacosaurus mongoliensis*¹⁴), and supplemented these data with histological growth series from the literature (*Syntarsus rhodesiensis*³, *Massospondylus carinatus*⁴ and *Maiasaura peeblesorum*⁷). We also generated a histological growth series for *Shuvuuia deserti*, a small, highly derived maniraptoriform theropod (Fig. 1)¹⁹. We assessed age from growth lines in histologically prepared specimens (Fig. 1) and obtained mass estimates through the application of scaling techniques (see Methods). A sigmoidal equation was used to model the growth of each species and least-squares regression analysis was used to fit the curves to these data. Exponential stage growth rates were converted to daily growth rates using the appropriate number of days in the Mesozoic era²⁰. A regression line was fitted to the maximum growth rates for the dinosaurs to enable comparisons with data from the literature for extant vertebrates^{17,18}.

Our analysis revealed that sigmoidal equations (Fig. 2) accurately describe the growth data for the six dinosaurs we tested ($0.885 \leq r^2 \leq 1.0$). Exponential-stage growth rates ranged from 3.4 to 14,460 g day⁻¹, with values positively correlating with increased adult mass (Fig. 3). No taxon substantially deviated from the general dinosaurian trend ($r^2 = 0.96$). The length of the exponential growth stages ranged from about 1 to 6 years (Fig. 2). The onset of somatic maturity occurred between the ages of 3 and 13 years, with values positively correlating with increased body size (Fig. 2).

This research brings us considerably closer to understanding dinosaur biology. It is now possible to quantify dinosaur growth and evaluate the fit of hypotheses regarding maximum dinosaurian growth rates; to address how some dinosaurs attained giant proportions; and to elucidate how and when extremely rapid growth evolved in avian dinosaurs.

Heated debates among palaeontologists and physiologists have revolved around whether the Dinosauria grew like extant reptiles scaled up to giant proportions²¹, like extant birds and/or mammals²², or had growth rates intermediate between these major groups¹¹. The results from our study suggest that none of these models is correct. All dinosaurs grew at rates more rapid than

those of extant reptiles, but as a whole they did not show rates intermediate between reptiles and birds/mammals, nor values equivalent to the latter (Fig. 3). Unexpectedly, our results show that growth in the Dinosauria was unique among major vertebrate groups with members showing rates below, equivalent to, or above typical mammalian/avian rates depending on the size of animal being considered (Fig. 3).

The regression equation for the Dinosauria allows quantitative predictions of dinosaurian growth rates and gross exploration of these data within and beyond the 15,000-fold range of sizes we studied. It is evident that the smallest dinosaurs, such as the dromaeosaurid *Microaptor zhaoianus*²³, with a body mass of ~220 g (on the basis of the mass of *Shuvuuia* and scaling of femoral length), would have grown at ~0.33 g day⁻¹, a rate double that of extant reptiles of comparable adult mass (Fig. 3). Somewhat larger dinosaurs (1–20 kg) grew at rates (1.3–21 g day⁻¹) approximating those of marsupial mammals (Fig. 3). Animals 100–1,000 kg grew at rates (93–786 g day⁻¹) typical of precocial birds, and those 1,500–3,500 kg grew like eutherian mammals (1,144–2,504 g day⁻¹; Fig. 3). Very large sauropods, such as *Apatosaurus* (~25,000 kg),

had growth rates similar to those of whales (absolutely and relatively some of the fastest-growing eutherians). For example, our analysis of a 25,952-kg *Apatosaurus* indicates a growth rate of 14,460 g day⁻¹, compared with 20,700 g day⁻¹ for a 30,000-kg gray whale (*Eschrichtius robustus*)¹⁷. Despite the amazing growth rates for these extinct terrestrial animals, the fastest-growing animal known is still the blue whale (*Balaenoptera musculus*) at 66,000 g day⁻¹ (ref. 17). The largest dinosaurs (for example, *Argentinosaurus*), tentatively estimated at 100,000 kg (ref. 24), are predicted to have grown at absolutely slower rates of 55,638 g day⁻¹.

Despite showing growth rates accelerated from the primitive reptilian character state, non-avian dinosaurs never attained extremely rapid rates like those seen in extant altricial birds (Fig. 3). For example, even the largest sauropods²⁴ would have grown at rates half that of a scaled-up altricial bird (123,025 g day⁻¹; Fig. 3).

So exactly when did dinosaurs attain their unique growth rates? The results for the basal dinosaur taxa (for example, *Syntarsus* and *Massospondylus*) combined with the cosmopolitan distribution of growth rates with respect to mass among our sample suggest that these rates may have been dinosaur apomorphies dating back about

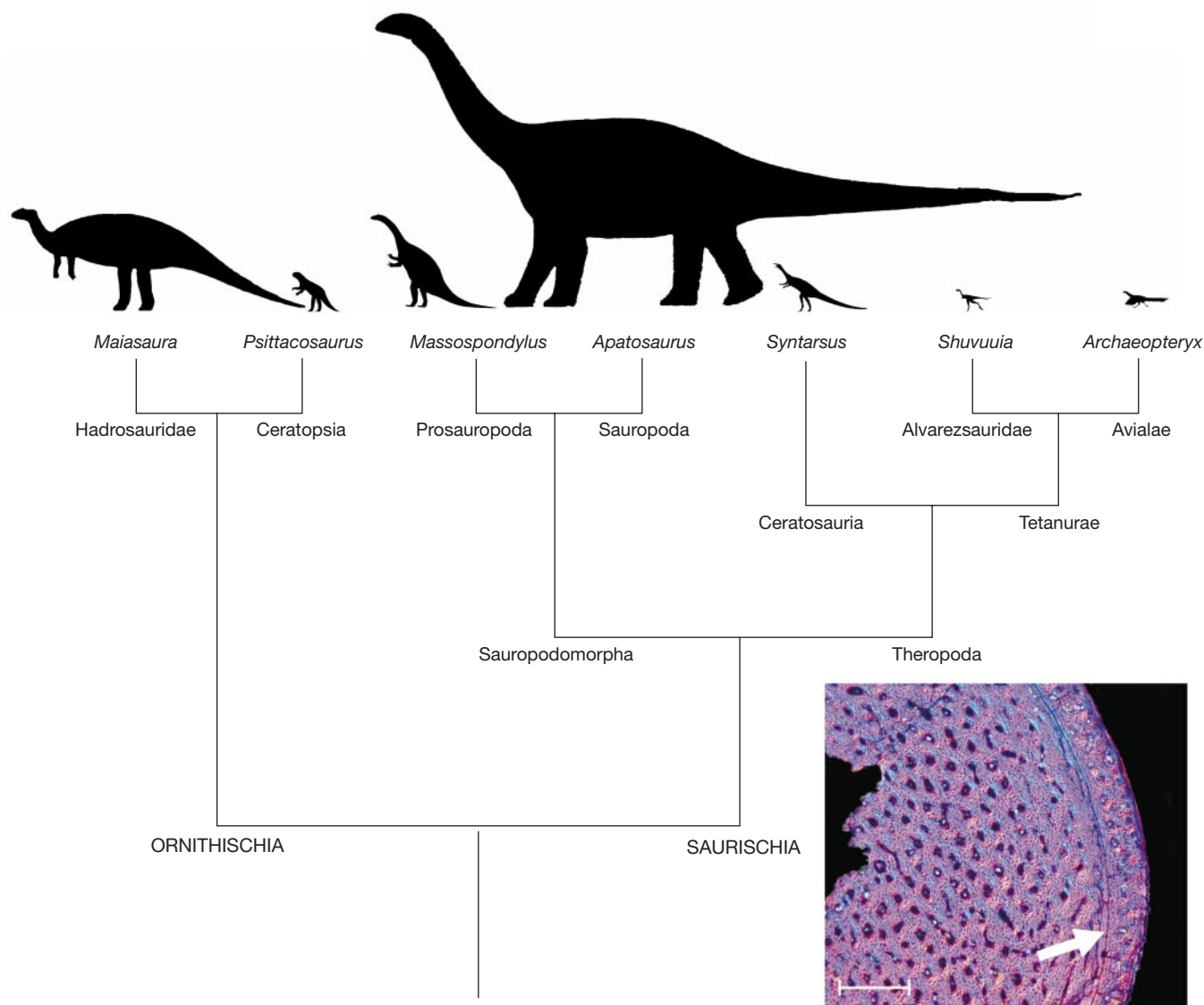


Figure 1 Cladogram for the Dinosauria showing the phylogenetic and size diversity of taxa represented. Six taxa, ranging in size from the 1.7-kg *Shuvuuia deserti* to the 26,000-kg *Apatosaurus excelsus*, were included. Recent phylogenetic analyses show *Shuvuuia*,

once thought to be a bird²⁹, to be a member of a sister clade to Avialae¹⁹. The inset is a histological section of a femur from *S. deserti* (AMNH 100/99) showing a growth line (arrow) used in the ageing of dinosaurs. Scale bar, 0.4 mm.

225 Myr. The first dinosaurs were bipedal forms about 25 kg that are predicted to have had growth rates 6.6 times faster than the primitive character state in reptilian predecessors (25.9 compared with 3.95 g day⁻¹). Whether the derived dinosaurian growth rates are a synapomorphy or if they evolved earlier in a closely related sister taxon (perhaps coincident with erect posture and associated physiological changes) is currently indeterminable.

Birds clearly attained a portion of their elevated growth rates from their dinosaurian ancestry, but when and how did they surpass

the rates of non-avian dinosaurs? Assuming the dinosaurian growth patterns hold true in other outgroups to the Avialae, this event occurred during or after² the evolution of birds. Key evidence for this hypothesis is regression data for small (0.22–20 kg) non-avian dinosaurs (including data for maniraptoriform sister taxa) showing that their growth rates were typically 2–7 times slower than those for similar-sized precocial birds (Fig. 3). Further, stepped evolutionary patterns of avian growth rates later occurred as rates soared fivefold or more above precocial levels and up to 75 times the original

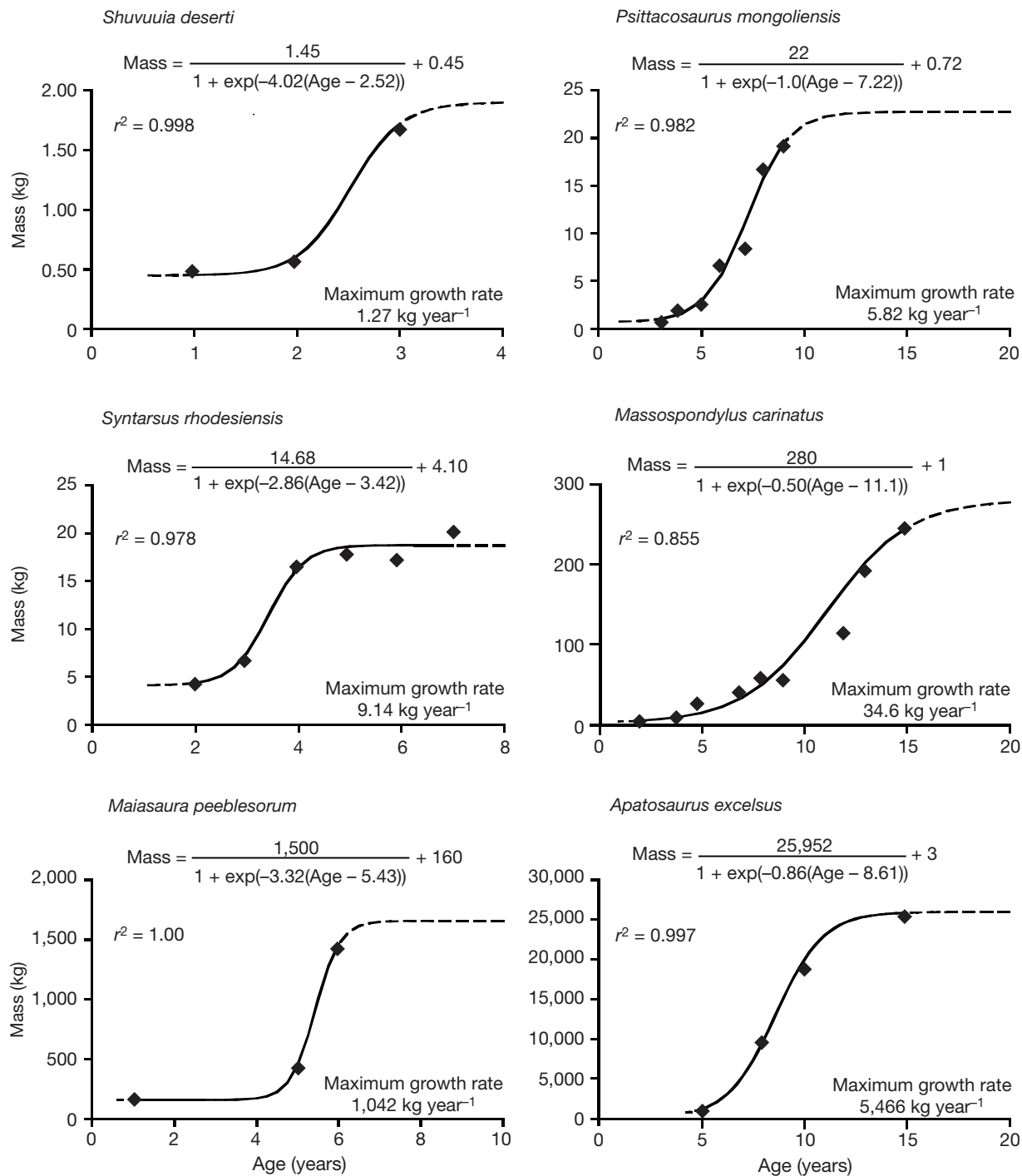


Figure 2 Growth curves for a diversity of dinosaurs. Dinosaur life history was characterized by a slow-growth lag stage during infancy. This was followed by an exponential growth stage midway through development, when maximum growth rates were obtained and most mass was accrued. Development culminated with a stationary

phase, when growth slowed or came to a standstill^{14,16}. Note that the largest animals in the growth series are among the largest specimens known for each taxon and the growth asymptotes were set accordingly¹⁴. The figure for *Psittacosaurus mongoliensis* is modified from Erickson and Tumanova¹⁴.

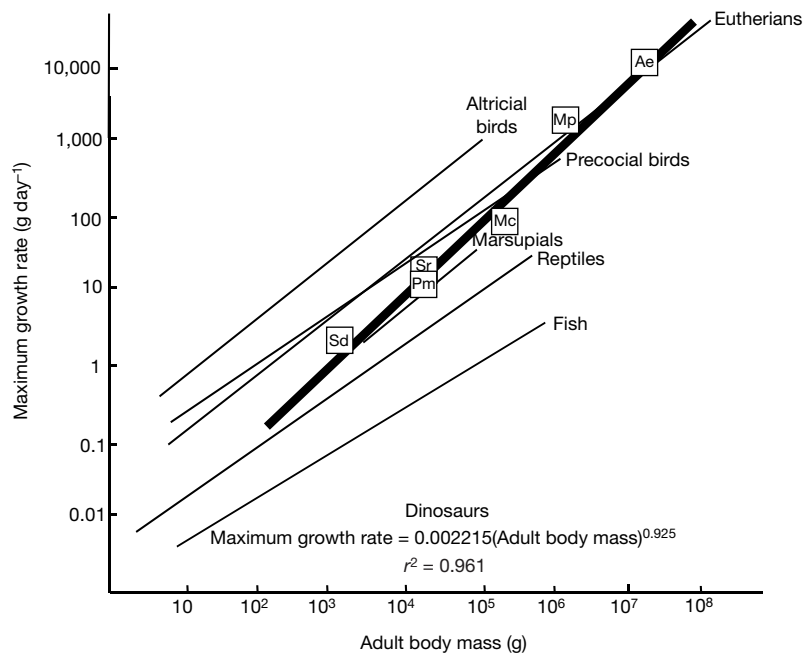


Figure 3 Comparison of exponential-stage growth rates in dinosaurs with typical values for extant vertebrates. Standardized comparisons are made using contrasts between animals of comparable adult mass to diminish signal from differences in shape and negate the effects of size^{17,18}. For example, growth of 20-kg reptiles (~3.5 g day⁻¹) can be compared to rates for 20-kg altricial birds (~270 g day⁻¹) to reveal a 75-fold difference in typical growth rates. The dinosaur regression line extends to the bounds of the known size range for the clade (~0.2–100,000 kg). Letters represent growth rates: Sd, *Shuvuuia deserti* (3.4 g day⁻¹); Pm, *Psittacosaurus mongoliensis* (12.5 g day⁻¹);

Sr, *Syntarsus rhodesiensis* (23.9 g day⁻¹); Mc, *Massospondylus carinatus* (90.3 g day⁻¹); Mp, *Maiasaura peeblesorum* (2,793 g day⁻¹); Ae, *Apatosaurus excelsus* (14,460 g day⁻¹). The steep slope for the Dinosauria is unique among major vertebrate clades but is not unprecedented for minor vertebrate clades spanning smaller ranges of body size¹⁷. Note that there is some overlap among groupings for individual taxa (not shown). For example, primates are extremely slow-growing eutherians, showing rates similar to those of marsupials¹⁷. Data for extant groups and graphics are modified from Case¹⁷ and Calder¹⁸.

reptilian condition in association with the evolution of altriciality^{17,18} (Fig. 3). □

Methods

Age assessment

Growth rings were counted in histological sections of each specimen²⁵. We accounted for loss of growth rings due to medullary expansion with increased age by sequentially superimposing subadult specimens upon those from larger individuals. The annual periodicity of these growth lines is established on the grounds of phylogenetic parsimony and tissue formation rates consistent with those for extant taxa^{14,25}.

Assessments of body mass

For all specimens except *Syntarsus*³ and *Massospondylus*⁴, body mass estimates were required. These were assessed using long-bone diaphyseal circumferences and regression equations from ref. 26. As these equations are invalid throughout development (that is, subadult animals of large taxa do not scale in proportion to adults of smaller taxa during ontogeny), we used developmental mass extrapolation¹⁴, a scaling principle like that developed in the middle of the last century²⁷. The accuracy of this methodology was tested for the purposes of the present study on human stature data for approximately 100 individuals²⁷ and on 34 wild alligators studied periodically throughout 8 years of development (G.M.E. and A. R. Woodward, unpublished data). Predictions of exponential growth rates were within 5% in each case (4.4% and 2.8%, respectively). This small degree of uncertainty and the general conformity (±50%) of our estimates of adult mass to other recent methodologies²⁸ suggest that the overall conclusions of this research are robust.

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Mealybug β -proteobacterial endosymbionts contain γ -proteobacterial symbionts

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Some insects have cultivated intimate relationships with mutualistic bacteria since their early evolutionary history. Most ancient 'primary' endosymbionts live within the cytoplasm of large, polyploid host cells of a specialized organ (bacteriome)¹. Within their large, ovoid bacteriomes, mealybugs (Pseudococcidae) package the intracellular endosymbionts into 'mucus-filled' spheres, which surround the host cell nucleus and occupy most of the cytoplasm². The genesis of symbiotic spheres has not been determined, and they are structurally unlike eukaryotic cell vesicles. Recent molecular phylogenetic and fluorescent *in situ* hybridization (FISH) studies suggested that two unrelated bacterial species may share individual host cells^{3,4}, and that bacteria within spheres comprise these two species⁵. Here we show that mealybug host cells do indeed harbour both β - and γ -subdivision Proteobacteria, but they are not co-inhabitants of the spheres. Rather, we show that the symbiotic spheres themselves are β -proteobacterial cells. Thus, γ -Proteobacteria live symbiotically inside β -Proteobacteria. This is the first report, to our knowledge, of an intracellular symbiosis involving two species of bacteria.

Most members of the large Hemipteran suborder Sternorrhyncha (aphids, whiteflies, psyllids, scales and mealybugs) feed on nutrient-deficient plant sap, and appear to fortify their diet with the metabolic products of mutualistic bacteria⁶. Mutualisms between insects and primary endosymbionts may date to the origins of the host families or superfamilies (100–250 Myr ago)^{7,8}. More recently acquired secondary endosymbionts are sometimes harboured in unspecialized syncytial or epithelial cells of the bacteriome^{1,8}, except in whiteflies, where two or more bacterial forms occupy the specialized host cells⁹. Endosymbionts are transferred vertically from maternal host cells to eggs *in vivo* in a highly organized process that reflects their antiquity¹. On the basis of molecular phylogenetic analyses of 16S ribosomal DNA, most insect endosymbionts have been identified as γ -subdivision Proteobacteria (purple bacteria), related to enterics such as *E. coli* and *Salmonella*¹⁰. Thus, the first report of mealybug endosymbionts as β -subdivision Proteobacteria (related to *Burkholderia*) was unusual⁴. *In situ*

hybridization of a γ -proteobacterial sequence to the bacteriome³ conflicted with that report⁴, but later seemed to be reconciled by the localization of both β - and γ -proteobacterial sequences to host cells⁵.

The peculiar mucoidal compartments within mealybug host cells have long puzzled researchers. Early studies detected RNA, ribosome-like granules, glycoproteins, microtubules and crystalline bodies in the mucus-like material of the spheres, but no cellular organelles¹¹. Protein synthesis within spheres (with a sphere and its bacteria treated as a unit) was independent from that of host cells¹². The ingestion of antibiotics by mealybugs, or exposure to high temperatures, resulted in degeneration of both bacteria and spheres within a few days; the insects died soon after¹³. Early electron microscopy revealed three membrane bilayers surrounding symbiotic spheres¹⁴; we note that this would be an unprecedented structure for eukaryotic cell vesicles.

We used polymerase chain reaction (PCR) and cloning to amplify and separate 16S rDNAs from bacteria present in bacteriome tissue of the citrus mealybug, *Planococcus citri* (Risso). Sequencing revealed similar numbers of clones from a β -subdivision proteobacterium (six) and a γ -subdivision proteobacterium (four). Quantitative PCR also suggested that numbers of both bacterial 16S gene copies in the bacteriome were similar—that is, neither result was due to a stray contaminant (amplification of both 16S genes most closely matched the 50,000 copy plasmid standards). The β -proteobacterial sequence was identical to a published bacterial sequence from *P. citri* (GenBank accession number M68890.2; originally as *Pseudococcus maritimus*)⁴. The γ -proteobacterial sequence was 93–94% identical to a tsetse fly secondary endosymbiont, a weevil primary endosymbiont, and several *Erwinia* spp.

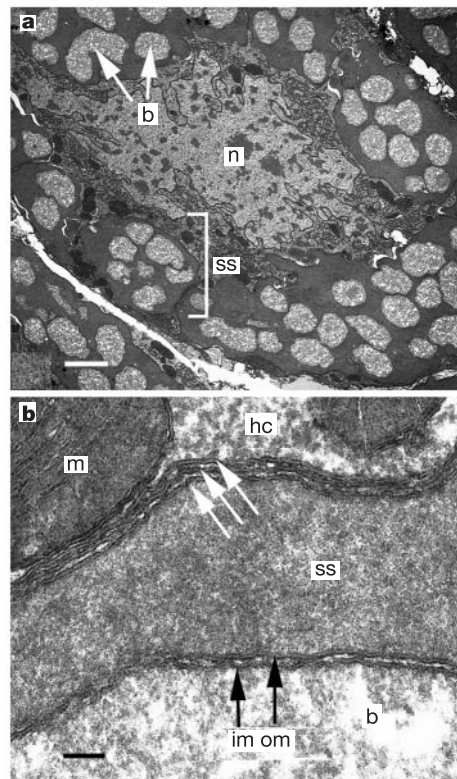


Figure 1 Transmission electron micrographs showing structure within specialized host cells. **a**, Host cell with central nucleus surrounded by seven spheres containing bacteria. Scale bar is 2.33 μ m. **b**, High magnification of two membranes of a bacterium (black arrows) and three membranes of a symbiotic sphere (white arrows). Scale bar is 0.0706 μ m. b, bacteria; hc, host cell cytoplasm; im, inner membrane; m, mitochondrion; n, nucleus; om, outer membrane; ss, symbiotic sphere.

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